

SEQUENCE LISTING



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<120> Modifications of Apoptin

<130> 2906-4996.1

<140> 10/083,849

<141> 2001-10-19

<150> US 60/242,397

<151> 2000-10-20

<160> 20

<170> PatentIn version 3.1

<210> 1

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MISC\_FEATURE

<222> (1)..(121)

<223> Apoptin (a small protein derived from chicken anemia virus) encoded by pCMV-Vp3 and by GFP-Apoptin constructs

<400> 1

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
 1 5 10 15  
 Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
 20 25 30  
 Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
 35 40 45  
 Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
 50 55 60  
 Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
 65 70 75 80  
 Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
 85 90 95  
 Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro  
 100 105 110  
 Arg Thr Ala Arg Arg Arg Ile Arg Leu  
 115 120

<210> 2

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> Apoptin protein encoded by pIRESneo alanine mutants

<220>

<221> MISC\_FEATURE

<223> Differs from Apoptin protein encoded by pCMV-vp3 and by GFP-Apopt  
 in constructs by replacement of the arginine residue at position  
 116 with a lysine residue

<400> 2

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Lys Arg Arg Ile Arg Leu  
115 120

<210> 3

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-86 of 5-alanine linker-scanning mutant series of Ap  
optin

<400> 3

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Ala Ala Ala Ala Ala Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
115 120

<210> 4

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-91 of 5-alanine linker-scanning mutant series of Ap  
optin

<400> 4

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
4



Arg Thr Ala Arg Arg Arg Ile Arg Leu  
 115 120

<210> 6

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-101 of 5-alanine linker-scanning mutant series of A  
 poitin

<400> 6

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
 85 90 95

Val Ser Glu Leu Ala Ala Ala Ala Ala Thr Thr Thr Pro Ser Arg Pro  
 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
 115 120

<210> 7

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-106 of 5-alanine linker-scanning mutant series of A  
poptin

<400> 7

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Ala Ala Ala Ala Arg Pro  
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
115 120

<210> 8

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-111 of 5-alanine linker-scanning mutant series of A  
poptin

<400> 8

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Ala Ala  
100 105 110

Ala Ala Ala Arg Arg Arg Ile Arg Leu  
115 120

<210> 9

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-116 of 5-alanine linker-scanning mutant series of A  
poptin

<400> 9

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Ala Ala Ala Ala Ala Leu  
115 120

<210> 10

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T106A of Apoptin

<400> 10

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Thr Thr Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
115 120

<210> 11

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T107A of Apoptin

<400> 11

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Ala Thr Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
115 120

<210> 12

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T108A of Apoptin

<400> 12

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Ala Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
 115 120

<210> 13

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant P109A of Apoptin

<400> 13

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Ala Ser Arg Pro  
 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
 115 120

<210> 14

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T106E of Apoptin

<400> 14

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Glu Thr Thr Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
115 120

<210> 15

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T107E of Apoptin

<400> 15

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Glu Thr Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
115 120

<210> 16

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T108E of Apoptin

<400> 16

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Glu Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
115 120

<210> 17

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutation T106A107A of Apoptin

<400> 17

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Ala Thr Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
115 120

<210> 18

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutant T107A108A of Apoptin

<400> 18

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Ala Ala Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
115 120

<210> 19

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutant T106A108A of Apoptin

<400> 19

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe  
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu  
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly  
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn  
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln  
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg  
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Thr Ala Pro Ser Arg Pro  
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu  
115 120

<210> 20

<211> 8

<212> PRT

<213> Chicken anemia virus

<220>

<221> MISC\_FEATURE

<223> amino acid sequence encoding the SV40-Large T nuclear localization signal

<400> 20

Pro Pro Lys Lys Lys Arg Lys Val  
1 5